NO-independent mechanism mediates tempol-induced renal vasodilation in SHR

Louise Tilma de Richelieu, Charlotte Mehlin Sorensen, Niels-Henrik Holstein-Rathlou, and Max Salomonsson

Division of Renal and Cardiovascular Research, Department of Medical Physiology, The Panum Institute, University of Copenhagen, Copenhagen, Denmark

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Tilma de Richelieu, Louise, Charlotte Mehlin Sorensen, Niels-Henrik Holstein-Rathlou, and Max Salomonsson. NO-independent mechanism mediates tempol-induced renal vasodilation in SHR. Am J Physiol Renal Physiol 289: F1227–F1234, 2005. First published July 20, 2005; doi:10.1152/ajprenal.00116.2005.—We investigated whether tempol, a superoxide dismutase mimic, affected renal hemodynamics and arterial pressure in spontaneously hypertensive rats (SHR) and Sprague-Dawley (SD) rats. We also examined whether tempol affected exaggerated renal vasoconstrictor responses to ANG II in SHR. To test whether the effects of tempol were due to a restored NO system, we used the NOS inhibitor Nω-nitro-l-arginine methyl ester (l-NAME). Renal blood flow (RBF) and mean arterial pressure (MAP) were measured in vivo by electromagnetic flowmetry and arterial catheterization in 10- to 12-wk-old anesthetized SHR and SD rats. Systolic arterial pressure (SAP) was measured in conscious rats using the tail cuff method. Tempol (1 mM) was given in the drinking water to SD (SD-T) and SHR (SHR-T) for 5–7 days for RBF measurements and for 15 days for SAP measurements. Age-matched SD (SD-C) and SHR (SHR-C) were used as controls. ANG II (1-4 ng) was administered as a bolus via a renal artery catheter. l-NAME was administered intravenously for 15–20 min. Renal vascular resistance (RVR) was elevated in SHR-C compared with SD-C. In SHR-T, baseline RVR was not different from SD-C and SD-T rats. Tempol had no effect on RVR in SD. l-NAME elevated RVR to the same extent in all four groups. Arterial pressure was not affected by tempol. The RVR responses to ANG II were higher in SHR-C than in the SD-C group. ANG II responses were not different between SHR-T and SD-T. Overall, tempol reduced the renovascular responses to ANG II in SHR. l-NAME elevated the effects of ANG II in SD-C rats but had no effect on the ANG II responses in the other groups. Thus l-NAME treatment did not influence tempol’s effects on baseline RVR or ANG II responses. We conclude that in SHR, tempol has a significant renal vasodilator effect and that it normalizes the increased renovascular ANG II sensitivity. As the effects of l-NAME are not greater in SHR-T rats, it is not likely that the elevated renal resistance and ANG II sensitivity in SHR are due to reactive oxygen species-induced quenching of nitric oxide.

angiotensin II; hypertension; nitric oxide; oxidative stress; renal hemodynamics; spontaneously hypertensive rats

KIDNEY FUNCTION IS of central importance in the control of normal blood pressure and in the development of essential hypertension (35, 36). Spontaneously hypertensive rats (SHR) have an elevated renal vascular tone compared with normotensive rats (7, 14, 24). Also, an augmented response to vasoconstrictors, among them ANG II, has been observed in the genetically hypertensive rats (8, 14, 15, 26, 50). Despite intensive research, the reason for this enhanced renal vascular tone and reactivity in hypertensive animals is still not resolved.

Elevated oxidative stress and high levels of reactive oxygen species (ROS) have been suggested to play a role in the pathogenesis of hypertension in human essential hypertension and in different hypertensive animal models, among them the SHR strain (1, 13, 23, 30, 32, 39, 40, 42, 43, 45). In support of this hypothesis, antioxidants such as ascorbic acid (vitamin C) have been found to reduce blood pressure in human hypertensive subjects (16). Elevated levels of superoxide ($O_2^-$) have also been observed in SHR (32, 43, 45, 47), and there is an increased production of $O_2^-$ in mesenteric arterioles and aortic rings from SHR (45, 49). Tempol treatment normalizes the $O_2^-$ concentration in this strain. In kidneys from SHR there is also an upregulation of mRNA subunits of NADPH oxidase, which has been identified as an important source of $O_2^-$ (6). It is also reported that $O_2^-$ causes contraction of aortic rings and that vessels from SHR are twice as sensitive as vessels from Wistar-Kyoto (WKY) rats (2).

The mechanisms by which the elevated $O_2^-$ levels result in elevated vascular tone and hypertension are not clarified. Much attention has been paid to the fact that $O_2^-$ inhibits endothelium-dependent vasodilatation (18, 38). This is supposedly due to the reaction between $O_2^-$ and nitric oxide (NO) to form peroxynitrite (ONOO−) (3). This, in turn, lowers the levels of NO, thereby causing vasoconstriction and hypertension (20, 23, 31, 42, 51). It has been proposed that such a mechanism may play a role in determining renal blood flow. The suggestion was based on the observation that the superoxide dismutase (SOD) mimetic drug tempol lowered the renal vascular resistance (RVR) and blood pressure in SHR (42). Also, in that study, when the hypertensive rats were pretreated with the NO synthesis inhibitor l-NAME, tempol had no effect on blood pressure, indicating that the effect of tempol is NO dependent. A similar effect of $O_2^-$ has also been suggested to play a role in ANG II-induced renal vasoconstriction (27). In that study, the investigators found that ANG II induced an NADPH oxidase-dependent production of $O_2^-$, which reduced the bioavailability for NO and in turn contributed to renal vasoconstriction. Direct measurements of rat afferent arteriolar diameter utilizing the blood perfused juxtaglomerular nephron preparation show that tempol causes vasodilation of this vessel in SHR but is without effect in WKY (20). It was suggested that $O_2^-$ selectively inhibited the action of neuronal nitric acid synthase (nNOS), which in the kidney emanates from the macula densa cells.
In the present study, we tested whether scavenging of O$_2^-$ by the membrane-permeable SOD mimetic tempol has a larger effect on basal RVR and on the RVR response to ANG II in SHR than in normotensive Sprague-Dawley (SD) rats; and second, whether an increased effect of tempol in SHR could be explained by a decreased availability of NO due to an increased quenching of NO by ROS in this strain. The latter hypothesis was tested by blocking the NOS with N-nitro-l-arginin methyl ester (l-NAME). In addition, we investigated the effects of tempol on the arterial pressure in anesthetized and conscious rats.

**METHODS**

The experiments were performed in 10- to 12-wk-old male SD rats (Møllegaard, Lille Skensved, Denmark) and 10- to 12-wk-old male SHR (Charles River, Sulzfeld, Germany).

All protocols were approved by the Danish Animal Experiments Inspectorate and experiments were performed according to national rules for the care and use of laboratory animals. All rats were fed ordinary rat chow (Altromin nr. 1314, Chr. Petersen, Ringsted, Denmark) containing 87 mmol Na$^+$/kg and had free access to tap water.

**Preparation of the Animals**

Four groups of rats were used in the study as follows: 1) SD control (SD-C); 2) SD tempol (SD-T); 3) SHR control (SHR-C); and 4) SHR tempol (SHR-T). Tempol (1 mM) was administered in the drinking water to rats of groups 2 and 4 for 5–7 days before the day of the experiment. Preparation of animals for surgery and measurements of renal blood flow were performed as previously described (41, 48). Briefly, anesthesia was induced by 8% sevofluran delivered in 35% O$_2$–65% N$_2$. Two polyethylene catheters were placed in the left jugular vein (PP-10) for infusion and in the right femoral artery (PP-25) for continuous measurement of the systemic blood pressure by a Statham P23-dB pressure transducer (Gould, Oxnard, CA). After a tracheotomy, the rat was placed on a servo-controlled heating table to maintain body temperature at 37°C. The rats were ventilated by a small-animal ventilator (tidal volume 1.7–2.3 ml, 60 breaths/min). The final sevofluran concentration needed to maintain sufficient anesthesia was ~2%. An intravenous bolus injection of the muscle relaxant pancuronium (0.6 mg Pavulon, Oranon, Holland) in 0.4 ml 0.9% saline was given, followed by continuous intravenous infusion of Pavulon (0.6 mg/ml) and saline (20 μl/min). The left kidney was exposed by a laparotomy extended to the left flank. The left ureter was catheterized (PP-10 connected to PP-50) to ensure free urine flow. The left renal artery was stripped of any fat or fascia. The left femoral artery was catheterized (tethered PP-10), and the catheter was carefully advanced through the abdominal aorta until the tip of the catheter was ~1–2 mm inside the left renal artery. The catheter did not interfere with RBF. ANG II (Sigma) was administered directly into the renal artery via the catheter. To prevent clotting, saline was infused (10 μl/min) through the catheter during the whole experiment.

**Measurement of RBF**

A precalibrated electromagnetic perivascular flow sensor (Scalar Medical, model 1401) was placed around the left renal artery (lumen diameter 0.6–0.8 mm). Blood pressure and RBF were sampled online utilizing a Powerlab 18 SP system (AD instruments, Chalgrove, UK) for later analysis on a PC. The kidney was superfused with warm saline (37°C) during the experiment. After preparation, the rat was allowed to equilibrate for 30 min before the experiment started.

**Experimental Protocol**

Three consecutive boluses of ANG II (1, 2, and 4 ng) were administered via the left renal artery catheter. One minute before ANG II injection, the saline infusion rate through the renal artery catheter was increased from 10 to 144 μl/min. The infusion rate allowed the ANG II bolus to reach the kidney within 9 s. A six-port injection valve (Upchurch Scientific, Oak Harbor, WA) was used to introduce the 10 μl ANG II bolus. RBF and arterial blood pressure were continuously recorded. When RBF returned to baseline, the infusion rate of saline was reduced to 10 μl/min. After a recovery period of 5 min, the next ANG II bolus was administered. When this protocol was completed, l-NAME (10 μg/kg, 18528 l/h) was administered intravenously with an infusion rate of 20 μl/min. After ~30 min of infusion, the experimental protocol was repeated.

**Measurement of Arterial Pressure in Conscious Rats**

Systolic arterial pressure (SAP) was measured in conscious rats using the pneumatic tail-cuff method (W+W Blood pressure reporter, model 8006, Ugo Basile) (10). SAP was measured in the same four groups of rats as in the series of RBF measurements (SD-C, SD-T, SHR-C, and SHR-T). Tempol (1 mM) was administered in the drinking water to SD-T and SHR-T rats for 15 days. The SD-C and SHR-C groups were used as time controls. Two consecutive measurements were recorded in a blinded fashion and averaged. Before the measurement, the rats were allowed an acclimatization period of 30 min in the measuring chambers. During the entire measurement period, the temperature inside the chambers was maintained at ~35°C. SAP was measured immediately before initiation of tempol treatment and every 3 days for the following 15 days.

**Data Analysis**

Data were presented as means ± SE. SigmaStat (Jandel Scientific/SPSS) software was used for data analysis. RVR was calculated as arterial blood pressure/RBF. Student’s t-test was used to test effects of l-NAME. One-way ANOVA followed by Student-Newman-Keul’s test was used for comparing the baseline values in the four groups of rats. Because of variance inhomogeneity when all four groups were compared, Kruskal-Wallis one-way ANOVA on ranks followed by Dunn’s test were used to compare the responses to ANG II between the four groups of rats. Repeated-measures ANOVA followed by Student-Newman-Keul’s test was used for evaluating the dose-response effects of ANG II and the measurements of SAP. Within the two strains of rats, the repeated-measures ANOVA included ANG II, l-NAME, and tempol as well as interaction terms between them as factors, with repeated measures on factors l-NAME and ANG II. A P value <0.05 was considered statistically significant.

**RESULTS**

**Effects of Tempol on Baseline MAP, SAP, RBF, and RVR**

**Control conditions.** The mean arterial pressure (MAP) measured in anesthetized rats was significantly elevated in the SHR-C group compared with the SD-C group (149 ± 3 vs. 118 ± 4 mmHg; n = 20 and 21, respectively). This difference persisted in the tempol-treated groups [143 ± 6 and 117 ± 5 mmHg for SHR-T (n = 10) and SD-T (n = 11), respectively]. There were no differences in MAP between the tempol-treated and control groups of the same strain. As a consequence of these negative findings, we further investigated SAP in conscious SD and SHR rats, utilizing the tail-cuff method. Before initiation of tempol treatment, SAP was 124 ± 4, 124 ± 5, 179 ± 7, and 165 ± 3 mmHg in SD-C (n = 8), SD-T (n = 8), SHR-C (n = 7), and SHR-T (n = 8), respectively. SAP was then measured every 3 days for the following 15 days. SAP was significantly increased in the SHR compared with the SD rats at all time points, but there was no difference between the tempol-treated and control rats of the same strain at any SAP
Effect of Tempol on RVR

EFFECT OF TEMPOL ON RVR

Measurement (Fig. 1). Furthermore, SAP did not change significantly over time in any of the groups.

In the SD-C group, RBF was 7.4 ± 0.3 ml/min, a value not different from 7.8 ± 0.3 ml/min found in the SD-T group. In contrast, tempol had a significant effect on baseline RBF in hypertensive rats. In the SHR-C group, RBF was 5.9 ± 0.4 ml/min, which is significantly lower than in the SD-C group. In the SHR-T rats, this value was restored to a value not different from that in the SD-C and the SD-T groups (7.6 ± 0.6 ml/min).

Calculating the RVR reveals a pattern similar to that of the RBF measurements (Fig. 2). Baseline RVR in SD-T was found to be unchanged compared with SD-C (15.5 ± 1.1 and 16.3 ± 0.6 mmHg·ml⁻¹·min⁻¹, respectively). Tempol treatment had an attenuating effect on RVR in the hypertensive rats. In the SHR-T group, baseline RVR was 20.0 ± 2.1 mmHg·ml⁻¹·min⁻¹. This value is not different from that in the SD-C and SD-T groups but is significantly reduced compared with the value calculated for the SHR-C group (27.1 ± 1.7 mmHg·ml⁻¹·min⁻¹).

After L-NAME treatment. Thirty-minute intravenous infusion with L-NAME (10 mg·kg⁻¹·h⁻¹) resulted in decreased RBF and elevated MAP and RVR in all four groups. The effects of L-NAME treatment in paired experiments are summarized in Table 1 and Fig. 3.

Also after L-NAME treatment, RVR was significantly smaller in the SHR-T group compared with the SHR-C group (Fig. 2). However, tempol had no effect on the response to L-NAME in both the normo- and the hypertensive groups. In all groups, there was a similar increase in RVR following L-NAME, as summarized in Fig. 3. If anything, there was a tendency for the increase in RVR following L-NAME to be higher in the SHR-C compared with the SHR-T group. However, this difference did not reach statistical significance. As L-NAME does not have a greater effect in the tempol-treated SHR, there is no indication that the effect of tempol should be mediated via diminished O₂⁻-induced quenching of NO.

Effects of Tempol on the Renal Response to ANG II

Control conditions. Infusion of an ANG II bolus into the renal artery caused a dose-dependent fall in RBF in all groups. As MAP was unchanged, there were corresponding increases in RVR. The magnitudes of the RVR responses for the different doses of ANG II are shown in Fig. 4. The renal responses to ANG II were significantly higher in the SHR-C group than in the SD-C group for all doses. After tempol treatment, there was no difference between the SD and SHR groups. The responses in the SHR-T, SD-T, and SD-C groups are all similar. Tempol treatment did not affect the RVR response to ANG II in the SD groups. However, there was a tendency to a reduced responsiveness to ANG II in the SHR-T group compared with the SHR-C group (Fig. 4). This difference was confirmed by ANOVA, which showed a statistically significant overall effect of tempol in reducing the responsiveness to ANG II in the SHR. Despite this, none of the individual means (ARVR) corresponding to the same dose of ANG II differed significantly between the SHR-C and SHR-T groups. These results indicate that tempol attenuates the responses to ANG II in hypertensive rats but that it has no effect on the responses in the normotensive rats.

After L-NAME treatment. Figure 5 summarizes the corresponding responses to ANG II following L-NAME treatment. The responses to ANG II were significantly elevated by L-NAME treatment only in the SD-C group, and only for the lowest dose (1 ng). In all other groups, the responses to ANG II were not significantly different from the responses before L-NAME treatment. In particular, in the tempol-treated SHR (SHR-T) group, the response to ANG II was not influenced by the L-NAME infusion. Tempol also significantly reduced the overall responsiveness to ANG II (ANOVA) during L-NAME.
treatment in SHR without having an effect on the individual doses. The effect of tempol on the ANG II responses was independent of the l-NAME treatment, as the interaction term between tempol and l-NAME (ANOVA) was statistically insignificant. It is therefore not likely that the elevated response to ANG II in the SHR-C group during control conditions (i.e., before tempol and l-NAME) is due to an inactivation of the NO system by ROS.

**DISCUSSION**

In the present study, we tested the hypothesis that scavenging of $O_2^-$ by the membrane-permeable SOD mimetic tempol, which catalyzes the conversion of $O_2^-$ to $H_2O_2$, has a larger effect on basal RVR and on the RVR response to ANG II in SHR than in normotensive SD rats. As $O_2^-$-dependent quenching of NO has been suggested to be involved in the pathogenesis of hypertension, we also examined whether an increased effect of tempol in SHR could be explained by a downregulation of the NO system due to quenching of NO by ROS (22, 23, 42). Furthermore, we investigated the effects of tempol on the arterial pressure in anesthetized and conscious rats. We quantified RBF in vivo in normotensive SD rats and in rats from the SHR strain.

ROS, among them $O_2^-$, are elevated in SHR compared with normotensive rats and this increase is reversed by tempol treatment (32, 43, 45, 47). Schnackenberg et al. reported that the urinary excretion of 8-iso PGF$_{2\alpha}$, a marker of oxidative stress, was increased in SHR. Two weeks of tempol treatment significantly decreased urinary 8-iso PGF$_{2\alpha}$, indicating a lowered production of $O_2$ (43). Utilizing lucigenin chemiluminescence, Park et al. (32) found that the $O_2^-$ concentration in aortic rings from stroke-prone SHR was decreased by 6-wk tempol treatment. Also, in afferent arterioles from SHR components of the $O_2^-$ generating NADPH oxidase, which is an important source of $O_2^-$, have been found to be overexpressed compared with the afferent arterioles from WKY rats (6).

In the present study, 5–7 days of treatment with tempol in the drinking water had no effect on MAP in anesthetized SD rats and SHR. We also treated SHR and SD rats with tempol in the drinking water for 15 days both to further investigate the long-term effects of tempol on arterial pressure and to rule out possible effects of anesthesia. Measurements of SAP were performed every 3 days in the conscious rats utilizing the tail-cuff method. We did not, at any point of measurement, find a significant difference between the tempol-treated and time control rats of the same strain. Also, the SAP value was never different from the initial value obtained before tempol treatment started. Our findings contrast, at least in part, results obtained by other investigators. For example, Yanes et al. (54) found that 2 wk of tempol administered via the drinking water caused a 22% reduction in MAP of SHR when measured in anesthetized rats. Schnackenberg et al. (42) found that an intravenous bolus of tempol reduced MAP by 28% in SHR. The effect of tempol in WKY was significantly smaller. However, the effects were significantly smaller in both strains when

<table>
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<tr>
<th>l-NAME</th>
<th>SD-C ($n = 12$)</th>
<th>SD-T ($n = 11$)</th>
<th>SHR-C ($n = 11$)</th>
<th>SHR-T ($n = 10$)</th>
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<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>119±5</td>
<td>136±7†</td>
<td>117±5</td>
<td>133±8†</td>
</tr>
<tr>
<td>RBF, ml/min</td>
<td>7.3±0.4*</td>
<td>5.7±0.5†</td>
<td>7.8±0.3*</td>
<td>5.8±0.4†</td>
</tr>
<tr>
<td>RVR, mmHg·ml$^{-1}$·min</td>
<td>16.7±1.0*</td>
<td>25.7±2.2†</td>
<td>15.2±1.1*</td>
<td>25.3±3.3†</td>
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Values are means ± SE. Baseline mean arterial pressure (MAP), renal blood flow (RBF), and renal vascular resistance (RVR) in anesthetized rats. *$P < 0.05$ vs. SHR-C before l-NAME. †$P < 0.05$ vs. before l-NAME.
the rats were treated with tempol for 7 days. In SHR, tempol attenuated MAP by 10%, and in WKY there was no effect. On the other hand, Kagiyma et al. (21) found that tempol administration into the lateral ventricle for 2 wk did not cause any change in MAP.

In the present study, basal RVR was ~75% higher in SHR-C than in SD-C. Five to 7 days treatment with tempol had no effect on RVR in SD, but in SHR it was lowered, such that the difference between the strains was abolished. The lack of effect of tempol in SD indicates that there is no major effect of oxidative stress on renal hemodynamics or blood pressure in this strain. The tempol-induced reduction of RVR in SHR is in agreement with observations by other investigators. Schnackenberg et al. (42) found that tempol given intravenously reduced the RVR by 29% in SHR. The effect of tempol on RVR in WKY was significantly smaller. RVR was not evaluated after long-term tempol treatment in the study by Schnackenberg et al. (42). However, in another report by the same authors, 2 wk of tempol administered in the drinking water did not cause significant changes in renal hemodynamics, although there were tendencies toward a decrease in RVR and an increase in GFR (43). In the present study, the RBF is significantly increased in SHR-T compared with SHR-C rats while the MAP is unchanged. This points to a primary effect of tempol on renal hemodynamics, resulting in a decrease in RVR independent of autoregulation.

We cannot fully explain the discrepant effects of tempol on blood pressure and RVR in the present study. One explanation may be a preferential influence of ROS on renal sympathetic innervation in SHR (see below). This might lead to an isolated effect on renal hemodynamics. In that regard DiBona and Sawin (12) recently found that acute renal denervation increased RBF and decreased RVR but did not affect MAP in SHR.

It is widely assumed that the mechanism behind the ROS-induced development of hypertension involves O$_2^-$ quenching of NO (18, 22, 23, 38, 42). In contrast to other investigators, we did not find support for the notion that the vasoconstrictor effect of O$_2^-$ is mediated via downregulation of the NO system. Elevated levels of NO should then be expected in the tempol-treated hypertensive rats (SHR-T), and thus L-NAME should have a greater effect in this group. However, this was not the case in the present study. Inhibiting the NO system with L-NAME increased the RVR in all four groups to the same extent. In fact, there was a tendency toward a greater rise in basal RVR in the SHR-C group than in the other groups. Thus, as the response to L-NAME is not enhanced in the SHR-T group, there is no indication that the NO system plays a more prominent role in this group than in the SHR-C group.

Using DETC, an inhibitor of superoxide dismutase, Majid and Nishiyama (29) assessed the effects of increased oxidative stress on renal parameters in dogs. They found that RVR increased 30% and RBF decreased 21% after inhibition of superoxide dismutase. During inhibition of NO with nitro-L-arginine, the renal responses to DETC were significantly augmented. These results indicate that superoxide is able to cause renal vasoconstriction even in the absence of NO. Thus a fall in NO availability does not seem to be necessary for the superoxide-induced renal vasoconstriction. Such a notion is supported by the observations of Racasan et al. (33), who found that NO blockade by nitro-L-arginine (L-NNA) had a greater effect on RBF and RVR in SHR than in WKY.

In accord with observations from other investigators, we found that the renal vascular responses to ANG II were elevated in the SHR-C group compared with the normotensive control group (SD-C) (8). Tempol treatment abolished this difference as the responses to ANG II did not differ between the SD-T and SHR-T groups. ANOVA showed a statistically significant overall effect of tempol in reducing the response to ANG II in the SHR both before and after L-NAME treatment. This result despite the fact that the responses to the individual doses of ANG II did not differ between the SHR-C and SHR-T groups. In contrast, tempol had no effect on the responses to ANG II in the SD rats, supporting the observation that there is no major renal effect of superoxides in this strain (see above). This is in accord with observations by Lopez et al. (27), who found that the RBF response to intrarenal infusion of ANG II was unaffected by tempol pretreatment in SD rats. Shastri et al. (45) found that tempol treatment caused a more pronounced reduction in the response to ANG II in aortic rings and mesenteric vascular beds from SHR than from SD or WKY rats. These observations indicate that O$_2^-$ augments, or partly mediates, the responses to ANG II in SHR. The latter is supported by observations that ANG II stimulation led to increased lucigenin chemiluminescence, indicating elevated production of ROS in response to ANG II (37, 45). In the study by Shastri et al. (45), inhibition of the NO system, by either L-NAME or endothelial denudation, abolished the effect of tempol on the ANG II response. This indicates that the effect
of O$_2^-$ is mediated via quenching of NO. Furthermore, in streptozotocin-treated diabetic rats, Schoonmaker et al. (44) found that the impaired NO modulation of the ANG II response in diabetic rats was restored by SOD treatment, indicating O$_2^-$ quenching of NO. In contrast, we found that the effect of tempol on the responses to ANG II was independent of whether the NO system was blocked by l-NAME. Thus the results of the present study indicate that the mechanism behind the O$_2^-$ induced elevated RVR at baseline, and the augmented responses to ANG II in SHR may not solely be explained by quenching of NO.

This raises the question of alternative mechanisms whereby O$_2^-$ may influence RVR. As mentioned above, tempol metabolizes O$_2^-$ to H$_2$O$_2$. In a recent study utilizing the blood-perfused juxtamedullary nephron technique, it was indeed shown that exogenous addition of H$_2$O$_2$ caused NO-independent dilatation of the afferent arteriole (5). Increased concentrations of H$_2$O$_2$ could thus potentially lead to vasodilatation and decreased RVR. Nevertheless, this issue is complex, and H$_2$O$_2$-induced vasoconstriction in SHR has also been reported (17).

It has also been suggested that ROS may induce phosphorylation of cytosolic phospholipase and stimulate arachidonic acid release in vascular smooth muscle cells (34). As arachidonic acid metabolites can act as vasoconstrictors in the kidney, this might be a possible mechanism leading to O$_2^-$ induced renal vasoconstriction (28).

Another possibility is that oxidative stress might lead to increased renal production of adenosine (9). The increased adenosine levels may, in turn, lead to pregglomerular vasoconstriction and elevated RVR (19). Chen et al. (9) found that treating kidney homogenate with the O$_2^-$ donor KO$_2$ led to an increased $V_{\text{max}}$ of 5’-nucleotidase and doubling of adenosine production. Furthermore, SOD caused a concentration-dependent reduction of 5’-nucleotidase. They also found that inhibition of SOD by diethylthio-carbamic acid caused renal vasoconstriction in vivo which was caused by elevated endogenous production of adenosine.

It has also recently been recognized that increased oxidative stress might lead to activation of the sympathetic nervous system (4, 25, 46, 47, 52, 53). Scavenging of ROS by a SOD mimetic might thus have more pronounced effects in hypertension, as it is suggested that the sympathetic nervous system is more active in hypertensive subjects (11). Shokoji et al. (47) reports that tempol significantly reduced renal nerve sympathetic activity (RNSA) in SHR and WKY. The reduction in RNSA of SHR was double the reduction in WKY, indicating a greater activity of ROS in SHR, which is in accord with the results of the present study. As the sympathetic nervous system modulates the responses to ANG II, there is also a possibility that tempol might attenuate the responses to ANG II via inhibition of O$_2^-$ induced stimulation of the sympathetic nervous system. There is, of course, the possibility that the increased activation of the sympathetic nervous system is due to increased quenching of NO by elevated levels of ROS. However, there are reports that support an NO-independent action of ROS on the sympathetic nervous system (4, 46, 52). Campese et al. (4) report that NO blockade did not abolish the reduction of RNSA elicited by intracerebral injection of tempol. This suggests that the effect of tempol is, at least in part, independent of NO. In another study by Shokoji et al. (46), it was noted that application of l-NAME did not affect the reduced RNSA following local application of tempol onto the renal nerve. On the other hand, the ganglion blocker hexamethonium abolished the tempol effect. In addition, Xu et al. (52) report that l-NAME did not affect the tempol-induced inhibition of RNSA in DOCA-salt rats. Furthermore, in the same study, tempol was without effect after treatment with hexamethonium. It was also shown that the O$_2^-$ levels were elevated in sections of vena cava or aorta from DOCA-salt rats. Topical application of tempol in vitro lowered these elevated levels (52). In a later study, they found that intravenous bolus administration of tempol to DOCA-salt rats did not affect O$_2^-$ levels in the vena cava or aorta. The blood pressure was, however, reduced (53). They concluded that the antihypertensive effect of tempol on DOCA-salt induced hypertension is due to direct sympathetic nerve inhibition (53). Taken together, these findings indicate an NO-independent effect of tempol on RNSA. At present, it is not possible to give a comprehensive explanation for the discrepancies between the different studies. It is possible that ROS affect renal hemodynamics via several different mechanisms and that a particular mechanism is more prominent under a certain experimental condition.

In conclusion, we found that long-term treatment with tempol has a significant renal vasodilatory effect in SHR while the renal vasculature of SD is unaffected, indicating elevated renal vasoconstriction by ROS in SHR. We also report that tempol abolishes the differences in ANG II sensitivity between SHR and SD rats. Blocking the NO system with l-NAME does not have a greater effect on baseline RVR or ANG II sensitivity in SHR-T than in SHR-C. Thus we could not verify that the NO system in SHR is downregulated due to quenching of NO by ROS. We cannot fully explain the mechanisms behind the effect of tempol on the renal hemodynamics in SHR. Furthermore, the literature in the field is not conclusive. However, there are several possible mechanisms other than ROS quenching of NO. Among them is ROS-induced activation of PLA or adenosine production. Furthermore, ROS may activate the sympathetic nervous system on the central or peripheral level. Also, an unspecific action of tempol cannot be totally excluded based on this study. Thus there is a need for further studies to resolve the mechanism by which ROS may cause elevated RVR in SHR.

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